

In Vitro and In Vivo Bactericidal Activities of Vancomycin Dispersed in Porous Biodegradable Poly(ϵ -Caprolactone) Microparticles

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Received 18 October 2004/Returned for modification 15 November 2004/Accepted 23 March 2005

Treatment of methicillin-resistant *Staphylococcus aureus* osteomyelitis requires a prolonged antibiotic therapy with vancomycin. Because of its weak diffusion, the in situ implantation of vancomycin could be interesting. The activity of vancomycin encapsulated in microparticles was evaluated in vitro and in vivo on rabbit osteomyelitis and showed a good activity compared to intravenous administration.

Vancomycin is used to treat methicillin-resistant *Staphylococcus aureus* (MRSA) infections (2, 5, 22). Bone infections are treated by parenteral administration of vancomycin, but it does not provide high local bone concentration due to the poor vascularization of the cortical bone and the low penetration of this drug. Moreover, this antibiotic presents nephrotoxicity, ototoxicity, and poor venous tolerance. A decrease of this systemic toxicity would be resolved by directly implanting antibiotic-loaded biomaterials into the infected bone. Vancomycin cements (1, 12) have proven their efficacy in treating bone infections, but the release of vancomycin in such associations was observed over a limited time period. The interest of other devices (8, 9) lies in the prolonged in situ release of vancomycin over a period of several weeks, avoiding administration by a central catheter (27, 28, 30). We propose development of a drug delivery system (DDS) using poly(ϵ -caprolactone) (PCL) (11, 14, 15, 17, 33) as the biomaterial with both biodegradable (24, 25, 29) and prolonged-release properties (18) and good immune tolerance (20, 21). The aim of this study was first to check the vancomycin stability in microparticles, to evaluate the in vitro bactericidal activity of vancomycin encapsulated in such microparticles, and to compare the in vivo bactericidal activity of vancomycin incorporated into microparticles to that of vancomycin administered intravenously on a rabbit model of osteomyelitis.

Poly(ϵ -caprolactone) microparticles (Union Carbide SA, Rungis, France) were prepared using a simple emulsion (oil/water) technique with one gram of PCL and 250 mg of micronized vancomycin (Lilly France SA) powder. Results of laser granulometer analysis showed that microparticles with a mean diameter of $216.3 \pm 66.0 \mu\text{m}$ were obtained.

Microparticles appeared as spherical particles with a riddled area resembling little craters by scanning electron microscopy.

Microparticle drug loading was indirectly detected in the external phase by vancomycin chlorhydrate dosage by UV spectrophotometer, and encapsulation efficiency was calcu-

lated. Microparticles were loaded with $49.6\% \pm 3.6\%$ of vancomycin (110 mg of vancomycin per g of microparticles).

In vitro studies of vancomycin release from microparticles were controlled using a previously described dissolution test (7, 10) with 200 mg of microparticles corresponding to approximately 22.06 mg of loaded vancomycin. The amount of vancomycin released into aqueous solution was first determined by an high-performance liquid chromatography (HPLC) dosage (3). The HPLC results are expressed as the mean cumulated percentage of drug released \pm standard deviation (SD) as a function of time. The second dosage method is a microbiological assay that permits estimation of the active amount of vancomycin released during the in vitro dissolution test (19). Results for vancomycin HPLC and microbiological concentrations were compared using Tukey's test.

We studied two strains: *S. aureus* ATCC 25922, susceptible to methicillin and vancomycin (MSSA), and *S. aureus* P9, resistant to methicillin but susceptible to vancomycin (MRSA). MIC of vancomycin was 1 mg/liter for both strains. Bactericidal activity was studied by producing killing curves with an inoculum of 10^7 CFU/ml and 2 mg of free vancomycin, 26 mg of vancomycin-loaded microparticles (i.e., 2.86 mg of vancomycin), and 2 mg of vancomycin added to 26 mg of unloaded microparticles. Two controls without antibiotic (one with free microparticles and one without microparticles) were performed. Bacteria counts were realized after 0, 6, 24, and 48 h.

The efficacy of vancomycin microparticles was investigated in a chronic osteomyelitis rabbit model (23). A corticotomy of the left superior tibial metaphysis was performed. A hemostatic compress impregnated with 10^7 CFU of MSSA or MRSA strain was implanted in the metaphysis. After 4 days, compresses were removed, and a surgical lavage was performed. A bacterial count (B1) in bone marrow was performed. Before treatment, the animals were randomly assigned to two groups, G1 and G2 (five animals per group). In G1, animals received an 11-day treatment with intravenous vancomycin twice a day (100 mg/kg/day), corresponding to 30 mg/kg in human. In G2, 40 mg of microparticles containing 4.4 mg of vancomycin was implanted without any further treatment for 11 days. At the end of the treatment, animals were sacrificed. A new bacterial

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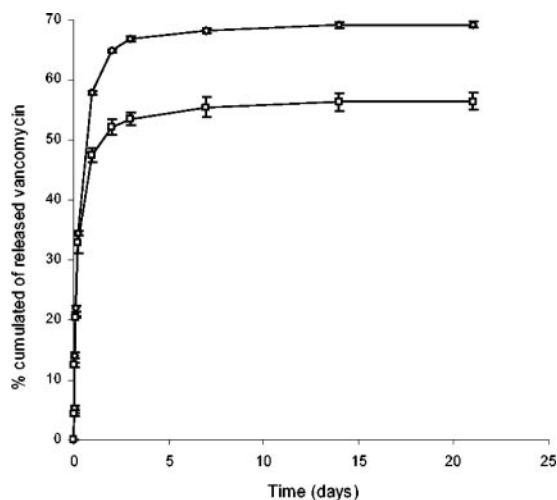


FIG. 1. Cumulated release rates of vancomycin after dissolution test. \diamond , microbiological assay; \square , HPLC assay. Error bars represent standard deviations.

count (B2) in bone marrow was performed. Bacteria were expressed in \log_{10} CFU/mg, and the difference of \log_{10} CFU/mg (B1 – B2) was calculated for each animal. The quantitative results were expressed as mean \pm SD. Analysis of variance (Statview; Abacus Concepts, Berkeley, CA) was used to compare the effects of the different groups, followed by a Scheffe test.

The release rate results of vancomycin obtained from HPLC and microbiological measurements are presented in Fig. 1. Vancomycin release occurred quickly up to 48 h and then was stable ($P < 0.001$). After 21 days, 56.4% of vancomycin was released from microparticles as evaluated by HPLC and 69.3% as evaluated by the microbiological test. Vancomycin concentrations were significantly higher with microbiological assay than with HPLC assay ($P = 0.08$).

Figure 2a (MSSA) and 2b (MRSA) show that the bactericidal activity of vancomycin dispersed in beads was similar for the two strains. Unloaded MP did not exhibit any intrinsic activity against *S. aureus* strains.

The bacterial count in bone was $6 \log_{10}$ CFU/mg before treatment. Decreases (B1 – B2) of $5.00 \pm 0.5 \log_{10}$ CFU/mg in G1 and of $4.10 \pm 0.5 \log_{10}$ CFU/mg in G2 were observed after treatment. The implantation of vancomycin dispersed in microparticles allowed significant killing in vivo after 11 days, and no difference between the two groups was observed ($P < 0.05$).

Treatment of MRSA osteomyelitis always requires a prolonged antibiotic therapy at least 6 weeks with vancomycin. The in situ implantation of antibiotic DDS could be a good option (18, 26) if the released vancomycin concentration is superior to the MIC during the first hours and can be maintained for several weeks. Vancomycin dispersed in microparticles showed a good bactericidal activity on the two strains, similar to that obtained with intravenous vancomycin or vancomycin added to unloaded microparticles.

This prolonged-release formulation allowed great reduction of the administered vancomycin dose and would limit the systemic administration and the renal toxicity. The in vivo test was performed during 11 days. Yenice et al. showed an antibiotic

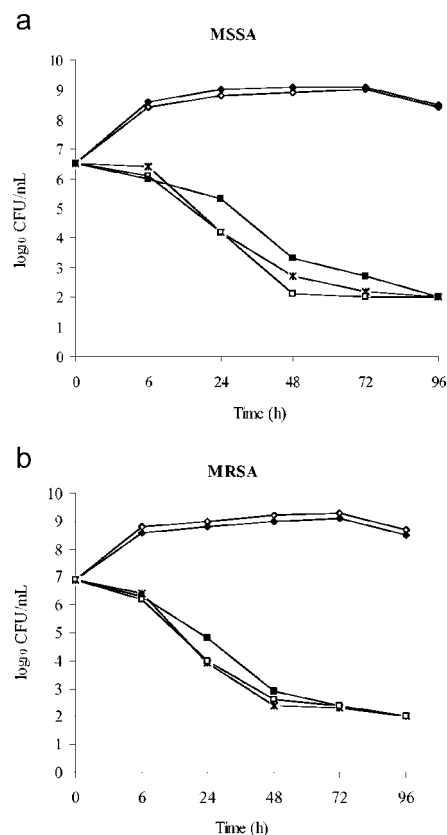


FIG. 2. Bactericidal activity of vancomycin alone and dispersed in microparticles. \diamond , control; \blacklozenge , control plus unloaded MP; \blacksquare , 218, loaded MP; \blacksquare , vancomycin plus unloaded MP; \square , vancomycin.

presence throughout 5 weeks in synovial sample (32). Nevertheless, it will be necessary to check the in vivo biodegradation of the poly(ϵ -caprolactone), as this polymer has a very slow degradation rate (6, 13, 24, 25) and could remain in the implantation site for a period greater than 1 year.

We plan in the future to associate these vancomycin microparticles to biphasic calcium phosphate granules (16) dispersed in a gel to form an injectable bone substitute (4, 31) to combine osteoconduction properties and therapeutic effects.

This study demonstrates the stability of vancomycin dispersed in poly(ϵ -caprolactone) microparticles and suggests that this DDS is a suitable vehicle for the delivery of high local concentrations of vancomycin in an implantation site. Vancomycin biodegradable microparticles could be used in implantation sites to avoid systemic side effects.

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